

# Podial Particle Picking in *Cassidulus caribaeorum* (Echinodermata: Echinoidea) and the Phylogeny of Sea Urchin Feeding Mechanisms

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**Abstract.** Selective particle-picking mechanisms of clypeasteroid echinoids (sand dollars and related taxa) are well-known. Those of the extant outgroup to clypeasteroids, the cassiduloids (lamp urchins), have not been analyzed to determine the origins of this sophisticated feeding mechanism. *Cassidulus caribaeorum* Lamarck, 1801, is a small cassiduloid living in the coarse, carbonate sands of protected beaches. The total gut contents of 24 specimens of *C. caribaeorum*, representing a full size range, were studied. The distribution of particle sizes in this sample was not significantly different from that of beach sediment, indicating that *C. caribaeorum* is probably not a selective deposit feeder. Juveniles with a test length of less than 3.5 mm do not feed, but all echinoids that are at least 5 mm long have full, or almost full, guts. The size of the mouth does not limit the sizes of particles eaten, regardless of the size of the animal. Allometric analyses suggest that podial size is also not a strong predictor of ingested particle size. *In vivo* and histological observations differentiate between the test cleansing functions of the spines and ciliary currents and the feeding activities of specialized podia. The new role played by accessory podia in food-collection by *C. caribaeorum* is a synapomorphy for cassiduloids and clypeasteroids, whereas the exclusive use of phyllopodia seen in earlier irregular echinoids is plesiomorphic.

## Introduction

The mechanism of particle collection by deposit-feeding members of the Clypeasteroidea (sand dollars, key-

hole urchins, sea biscuits, and allies) has been well studied in recent years (Ellers and Telford, 1984; Telford *et al.*, 1985, 1987; Telford and Mooi, 1986) and is well enough understood that a stochastic model has been developed and used to provide a computer simulation of the process (Telford, 1990). Particles are drawn from the sediment one by one, by foraging podia (or tube feet), and are then passed from podium to podium to reach the food grooves (shallow, ditch-like depressions in the test surface that lead from the distal regions of the test to the mouth). Stubby podia within the grooves then direct the material, which is usually gathered into a mucous cord, to the mouth. Although the collection of particulate material by suckered podia occurs in other echinoid groups, no research has specifically addressed the evolution of the mechanism.

Cassiduloids (lamp urchins) are more closely related to the Clypeasteroidea than to any other extant echinoid group (Mooi, 1990a; Suter, 1994), and would therefore be the most suitable outgroup for a comparative and evolutionary study of clypeasteroid feeding mechanics. Of the 30 or so living cassiduloid species, 5 occur in American waters (Mooi, 1990b). Only a single recent paper (Gladfelter, 1978) has described the biology of the commonest American cassiduloid, *Cassidulus caribaeorum* Lamarck, 1801. The feeding mechanisms in other cassiduloids have only been cursorily investigated. For example, Thum and Allen (1976) suggested that both "oral podia" and circumoral bourrelet spines lift particles into the mouth of *Echinolampas crassa*. Some selectivity was implied by their comments that larger particles were excluded from the diet because of the fixed size of the peristome, and that smaller particles were not eaten because the echinoid had difficulty manipulating them. Mooi

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(1990b) summarized what little is known of the biology of cassiduloids, showing that a comprehensive picture of feeding biology in the group awaits further study.

In this paper we describe the gross morphology and histology of every structure involved in feeding, thereby distinguishing them from the structures used in cleansing of the test. In doing so, we provide the only detailed description of all spine types and nonrespiratory podia found on a cassiduloid. We also perform the first allometric study of podia in an echinoid, concentrating on those podia involved in food-gathering and handling. On the basis of these observations, we describe the feeding mechanism of *Cassidulus* and contrast it with that of clypeasteroids. A detailed analysis of ingested material includes a statistical test for selectivity by this deposit-feeding urchin. Finally, we synthesize all these new data in a discussion of the evolution of phyllopodial feeding within the extant Irregularia (Holectypoida, Spatangoida, Holasteroida, Cassiduloida, Clypeasteroida) and an exploration of the origins of the sophisticated podial particle-picking mechanism (PPPM) in the scutelline sand dollars.

## Materials and Methods

### *Specimen collection*

Specimens of *C. caribaeorum* were collected at Loblolly Bay, on the resort island of Anegada, British Virgin Islands. The urchins occur 10–100 mm beneath the surface of the sediment in a small berm at the water's edge, at depths ranging from 0.5 to 1 m. They were collected by hand and transported live to a temporary laboratory for *in vivo* observation. Other specimens were fixed for 24 h in 2% glutaraldehyde in seawater, and stored in 70% ethanol. Specimens for podial measurements were first relaxed by dropwise addition of ethanol until no response to touch was observed.

### *In vivo observations*

Feeding was observed by viewing specimens with a stereomicroscope focused on an inclined, front-silvered mirror mounted at 45° beneath a small aquarium. The aquarium floor was sparsely covered with native sand from Loblolly Bay. Ciliary currents were visualized from both oral (bottom) and aboral (top) surfaces. A suspension of India ink or finely ground carmine particles in seawater was introduced between the spines *via* a hypodermic syringe, using the methods of Telford *et al.* (1985). On the aboral surface, these suspensions were added to areas around the apical system. On the oral surface, they were placed in areas adjacent to the mouth, and just below the ambitus (the edge of the test).

### *Histology*

Specimens were preserved in 10% formalin in seawater, or 2% glutaraldehyde in seawater passed through a Millipore filter. The following regions of the test and associated appendages were then sampled: ambulacrum I adjacent to the peristome, ambulacrum III on the oral surface, the locomotory area in ambulacrum 1, the naked zone in interambulacrum 5, and the anal sulcus (the deep groove in the test leading from the periproct, or anal region, to the posterior edge of the test). Using the methods outlined in Mooi (1986a), we decalcified the samples and then embedded them in epon araldite. Blocks were then sectioned at 0.5–1.0  $\mu\text{m}$  intervals and the sections affixed to microscope slides in series. The epon araldite was removed in a saturated solution of sodium hydroxide in 95% ethanol, and stained with thionin, toluidine blue, or Milligan's trichrome according to techniques described in Humason (1962). Sections were observed with a compound microscope and drawn with the aid of a camera lucida. Additional information about the morphology of spines and the positions and lengths of ciliary bands was gathered by observing "living" spines that had just been removed from the test and placed in seawater on well slides, or by slightly staining whole mounts of preserved spines with toluidine blue.

### *Test and podial measurements*

Test length, width, and height, and peristome length and width were measured to the nearest 0.1 mm with electronic calipers. The diameters of podial suckers on preserved specimens were measured with a calibrated eyepiece micrometer. Before the podia could be measured, they had to be made more visible, and this was accomplished by briefly staining the entire urchin in 1% methylene blue. For ambulacra III, IV, and V, every podium that was visible while looking directly at the oral surface was measured. When podia in ambulacra IV or V (called the paired ambulacra because they are almost mirror images of ambulacra II and I, respectively) were missing or obviously regenerating, measurements were obtained from the corresponding podia in the ambulacra on the other half of the test.

### *Sediment and gut content analyses*

Five samples of subsurface sediment were collected by hand from among the population of *C. caribaeorum* in the small berm at Loblolly Bay described above. Care was taken to collect the sediment samples from regions in the berm that contained the highest concentration of urchins. Small waves lapping against the berm kept the sediment constantly stirred, and stratification within the berm was not observed. Particles were strewn on glass slides and examined under a compound microscope

equipped with a camera lucida. A digitizing tablet (Houston Instrument Hipad) was positioned adjacent to the microscope for simultaneous viewing. For each particle of sediment, the two longest dimensions orthogonal to each other were obtained by digitizing. The mean dimension was calculated, and a computer program sorted the particles and assigned them to size classes at 100- $\mu\text{m}$  intervals (Telford, 1990). Twenty-four specimens ranging from 3.7 to 26.8 mm were dissected and their gut contents removed for individual analyses. The number of particles in the gut of each individual was counted under a dissecting microscope, and every particle was digitized as described above.

### Statistical procedures

All regressions were calculated by least squares fit of log-transformed data. An exponent ( $b$ ) of 1.00 therefore indicates isometry of the two variables, and the coefficient ( $A$ ) is the value of the dependent variable when the independent variable is 1.00.

The significance of the differences between the frequency distributions of particles from urchin guts and Loblolly Bay sand was tested using a log-likelihood ratio test ( $G$ -statistic) (Sokal and Rohlf, 1981):

$$G = 2 \sum O_i \ln (O_i/E_i)$$

where  $O_i$  is the observed frequency in the  $i$ th class and  $E_i$  the expected frequency. The null hypothesis,  $H_0$ , states that there is no difference between gut contents and surrounding sediment. The  $E_i$  values were obtained by multiplying the total number of particles in each gut sample by the proportions,  $p_i$ , of the particles in the surrounding sediment.

A stochastic algorithm was written to generate simulations of gut contents based on a random selection of particles. The probability of a foraging podium encountering a particle of any given size class depends on the contribution which that size class makes to the planar area of the sediment (Telford, 1990). This is estimated as:

$$A_i = (O_i * (M_i)^2) / (\sum O_i * (M_i)^2)$$

where  $A_i$  is the contribution to planar (or projected) area,  $O_i$  is the particle frequency, and  $M_i$  the mean particle dimension in the  $i$ th size class. Numbers between 1 and 100,000 were assigned successively to the particle size classes, according to their proportional representation. To simulate gut contents, random numbers were generated (seeded on the computer clock) and particles were added to the corresponding size classes. For each specimen, the observed number of particles in the gut determined the number of random particle selections to be made. Ten replicates were run for each specimen and for Loblolly Bay sand, and the means and SD were calcu-

lated. Frequency distributions were tested by the  $G$ -statistic. In a process analogous to bootstrapping, 10,000 random simulations of the pooled gut contents were made, and the distribution of the resulting  $G$ -statistics was examined.

## Results

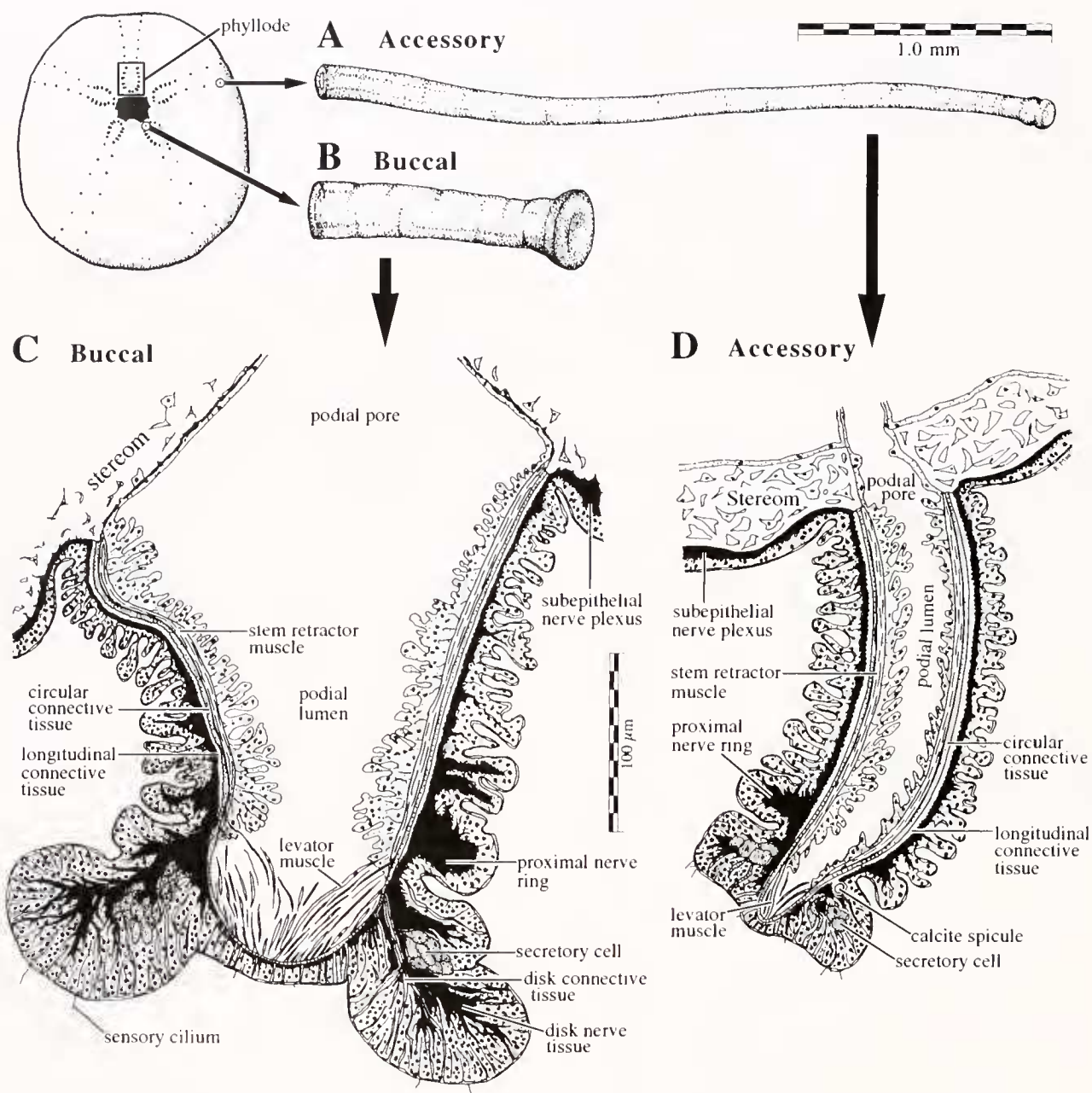
### Podial morphology

Four types of podia are found in the ambulacra of *C. caribbaearum*: respiratory podia, buccal podia, phyllopodia, and accessory podia. Respiratories are flattened, leaflike podia mounted on paired pores that continue to the apical system in two rows. These podia have very thin walls with reduced musculature. They lack attachment disks, and do not function as manipulatory structures. Respiratory podia are not involved in feeding and so are not treated here, except to note modifications of ciliary currents that enhance their efficiency in gas exchange (below). The other three types of podia—nonrespiratory—are well-muscled, thick-walled tubes terminating in a distinct terminal disk. Figure 1 illustrates the general appearance and histology of two of these three types. Each of these podia is mounted on a single pore that passes through the test to an internal saclike ampulla which is, in turn, connected to the lateral and radial water vessels in the typical echinoid manner. There is generally one podium per plate in the ambulacra. However, one plate in each pair of ambulacral basicoronals bears two podia: a buccal and a phyllopodium.

Each ambulacrum has a pair of buccal podia. Buccals are the first podia in the ambulacral series, and are located adjacent to the peristome, one in each ambulacral basicoronal plate. They are also the first podia to appear during development after the primordial podia (the latter comprise the "terminal tentacles" in the ocular plates). Even in adults, buccals are not very extensible (Fig. 1B), and are seldom capable of reaching more than a millimeter from the surface of the test. Frequently exceeding 300  $\mu\text{m}$  in diameter in adults (Fig. 1C), the terminal disk is greatly expanded and more than twice the diameter of the stem when the podium is extended.

Phyllopodia can also exceed 300  $\mu\text{m}$  in disk diameter, but tend to be somewhat smaller than the buccals in a given individual. Phyllopodia are very similar to buccals in having expanded disks, but are capable of greater extension. In each ambulacrum, there are two slightly curved arcs of phyllopodia which together form a structure known as the phyllode. In adults, each arc of the phyllode, composed of 5 or 6 phyllopodia, continues distally from a corresponding buccal podium (Fig. 1). The number of phyllopodia increases with size of the individual echinoid, and the podia tend to decrease in disk diameter with distance from the peristome.

Distal to the phyllodes are the accessory podia. These



**Figure 1.** Podia of *Cassidulus caribaeorum*. (A) Appearance in life of fully extended accessory podium. (B) Appearance in life of fully extended buccal podium. (C) Longitudinal section showing histology of retracted buccal podium. (D) Longitudinal section showing histology of retracted accessory podium. View of oral surface in upper left indicates locations of podia in A–D. In C and D, the scale bar is corrected for tissue shrinkage during histological preparation.

are highly extensible, with slender stems that can reach more than 3 mm beyond the test surface (Fig. 1A). The diameter of the disk (seldom exceeding 150 µm) is not much greater than that of the shaft, even when the accessory podium is fully extended and the wrinkles in the epithelium (Fig. 1D) are smoothed out. A single accessory occupies each ambulacral plate, making two rows per ambulacrum until they give way to the closely spaced

respiratory podia that constitute the lanceolate, gill-like structures on the aboral surface known as the petaloids.

#### *Podial histology*

The anatomy and histology of the buccal and accessory podia are summarized in Figure 1C and D. The histology of phyllopodia is very similar to that of buccal po-

dia, so they are not figured. The water vascular system extends into the stem to form a coelomic podial lumen lined with epithelium. There is a series of stem retractor muscles between this epithelium and a connective tissue sheath. These retractors insert around the outer rim of the podial pore and run up the shaft inside the connective tissue sheath to a point just proximal to the blind end of the sheath. The sheath consists of longitudinal and circular collagenous elements that stained vivid green in Milligan's trichrome; the fast green in this trichrome stain is specific for connective tissue. Inside the distal part of the connective tissue sheath is a series of levator muscles, each fiber of which arises from the inside of the blind end of the sheath and inserts near the point at which the stem retractors end. Just outside the connective tissue sheath is a subepithelial nerve plexus confluent with that overlying the test. The plexus thickens to form a nerve ring just proximal to the disk, and also gives rise to ramifications in the epithelium of the disk itself. The disk is made up of greatly thickened epithelium containing both ciliated and unciliated cells, secretory cells, and fibers of connective tissue arising from the connective tissue sheath. The central part of the disk supports short cilia, and the more peripheral parts of the disk tend to bear somewhat longer cilia. Within the epithelium of the central part of the disk are support cells bearing internal filaments that stain red in Milligan's trichrome. The acid fuchsin usually indicates the presence of muscle fibers, but in this case the red color seems to be resident in tonofilament bundles in the support cells (*sensu* Flam-mang and Jangoux, 1994). There is an abundance of secretory tissue in the peripheral parts of the disk epithelium, most of which stains purplish or dark blue in thionin and toluidine blue. The histological procedures used here were sufficient only to reveal the presence of secretory cells, but not the intracellular features that distinguish the various types.

The histology of all three types of nonrespiratory podia is very similar. The buccals and phyllopodia are the most alike, but the accessories tend to have fewer levator muscles, a narrower podial lumen, and much smaller disk and associated structures. Calcareous elements, when they are present at all, are found only in the accessory podia (Fig. 1D), never in phyllopodia or buccals. These spicules are small, rod-shaped elements embedded in the outer layers of the connective tissue sheath at the proximal part of the disk near the point at which the stem retractors end. Even in the accessory podia, spicules are extremely rare and when present, there is usually only one in any given podium.

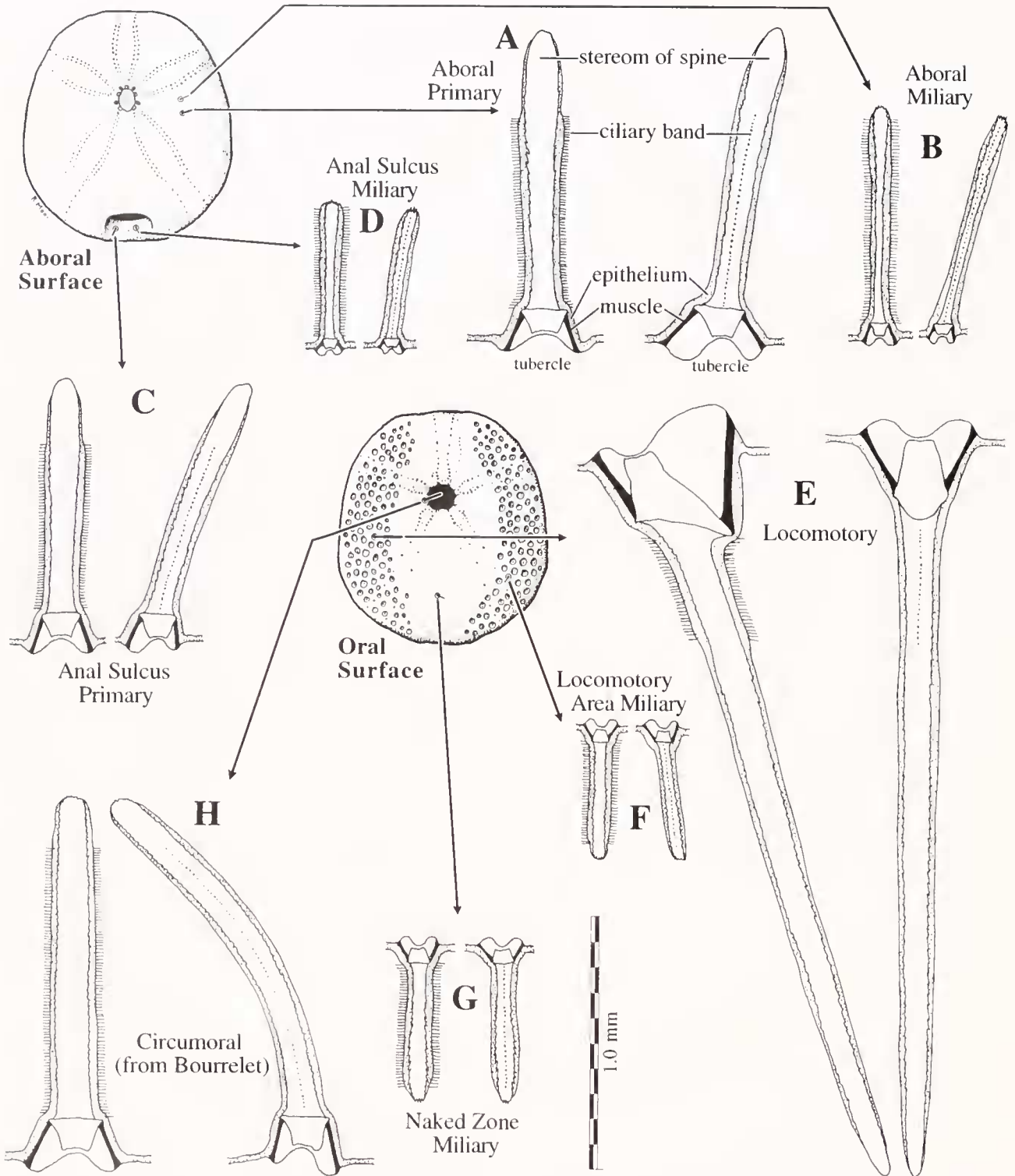
#### *Spine morphology and ciliation*

Two general types of spine can be found on the test of *C. caribaeorum*. Primary spines (*sensu* Mooi, 1986c) are

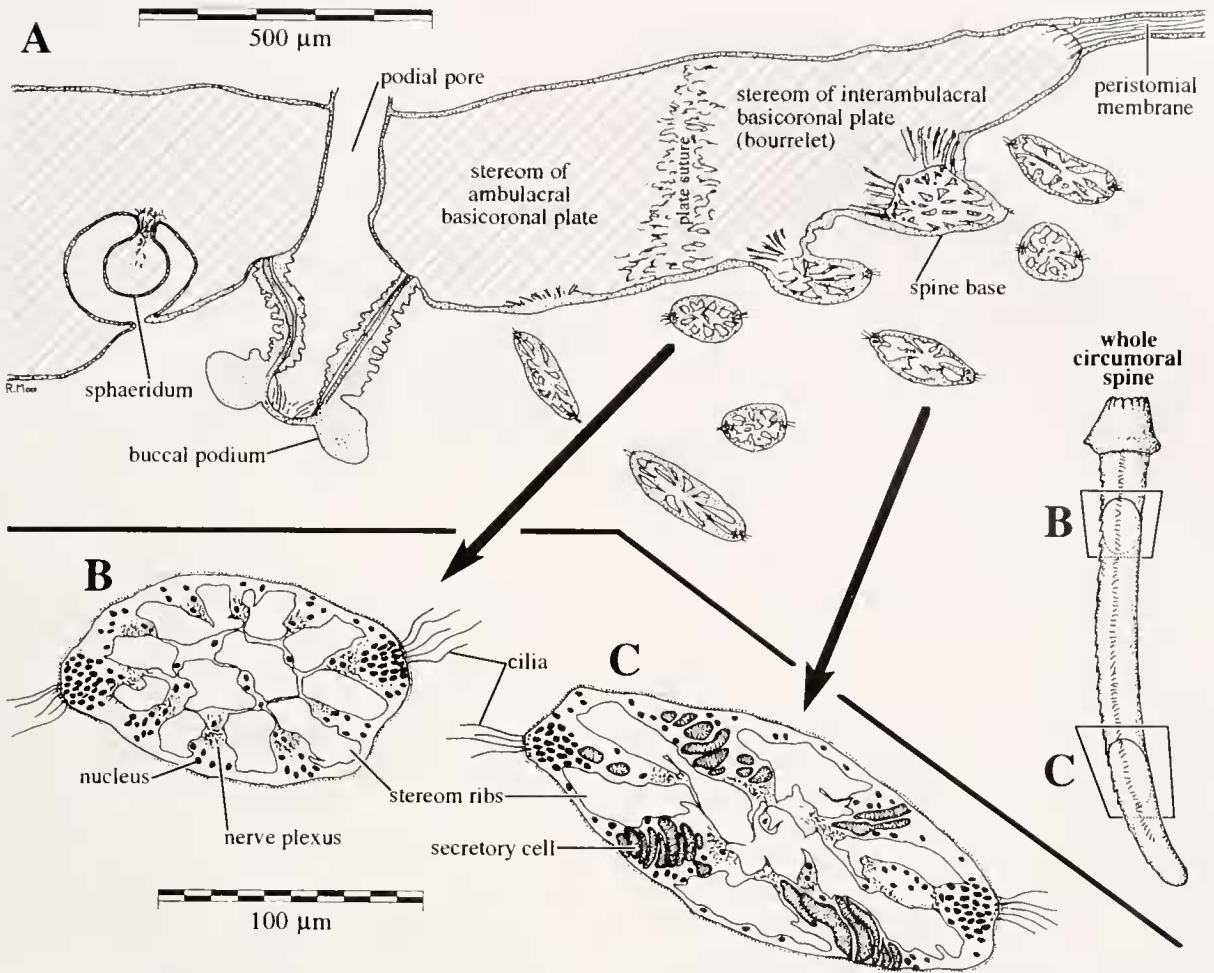
the longer type, occupying the largest tubercles on both aboral and oral surfaces. Miliary spines occupy the smaller tubercles interspersed among the primary tubercles. We distinguish among four major types of primary and four types of miliary spines in *C. caribaeorum* based on dimensions and curvature of the shaft, ciliary pattern, and position on the test (Fig. 2).

The longest spines on the top of the echinoid are known as aboral and anal sulcus primary spines (Fig. 2A and C respectively). The aboral primaries are slightly swollen distally, and distributed evenly over the aboral surface to form a layer of almost constant depth. Aboral primaries are generally slightly longer than the anal sulcus spines. The latter are known only from the anal sulcus, a shallow trough that extends posteriorly from the periproct (Fig. 2). On both these primary types, two ciliary bands run from the base of the spine distally for about 75% of the spine's length. As discussed below, the ciliary bands are located on opposite sides of the shaft in the pattern described by Mooi (1986c). Aboral and anal sulcus miliaries are found in the same areas (respectively) from which aboral and anal sulcus primaries are known. In each case, they are only about 75% the length of their corresponding primary spines, but the ciliary bands extend all the way to their ends (Fig. 2B, D). The miliary spines of some scutelline clypeasteroids terminate in balloonlike sacs consisting of swellings covered by epithelium and filled with collagen fibers (Mooi, 1986c), but the miliaries of *C. caribaeorum* lack distal swellings of any kind. All the spines on the aboral surface are slightly bent where the shaft meets the base so that the spine leans at a slight angle downslope from the apex of the test.

On the oral surface, there are two types of primary spines. Locomotory spines occupy two broad locomotory areas, one on each side of the oral surface near the edge of the test (Fig. 2). Each locomotory spine has a strong bend where the shaft meets the base so that the spine bends towards the midline of the oral surface. Ciliary bands extend from the base for about 20% of the length of the spines (Fig. 2E). Locomotories are the longest spines on the echinoid, occasionally exceeding 3 mm in length on adults more than 25 mm in test length. However, these spines vary slightly in length, with the longest in the centers of the locomotory areas and the shortest at the peripheries of the areas. Interspersed among the locomotories are very short miliaries that are among the shortest spines on a given specimen (Fig. 2F). Like the aboral miliaries, ciliary bands run almost the entire length of the spine. There are no primary spines in the so-called naked zone, which is populated only by sparsely distributed miliary spines that are similar in ciliation to, but slightly longer and more robust than, those in the locomotory area (Fig. 2G). In *C. caribaeorum*, the slight bulging of the interambulacral basiconical plates intrudes into the peristomial region cre-



**Figure 2.** Morphology and ciliation of spines of *Cassidulus caribaeorum*. Arrows indicate site of origin for each spine type: A–D from aboral surface; E–H from oral surface. For A–H, each pair of drawings shows two orthogonal views of same spine. Shading conventions used for labeled structures in A apply to all drawings in A–H. Scale bar refers to all spines in A–H. On drawing of oral surface, naked zone is outlined by fine, dotted lines.



**Figure 3.** Histology near peristome of *Cassidulus caribaeorum*. (A) Radial section of peristomial area adjacent to, but not through, perradial suture of ambulacrum I. Plate suture is between ambulacrum and interambulacrum. (B) Slightly oblique cross-section through proximal part of circumoral spine. (C) Slightly oblique cross-section through distal part of circumoral spine. Sections in B and C correspond to those shown in whole circumoral spine on right.

ating a “bourrelet.” On these bourrelets are series of densely packed, strongly curved circumoral spines (Fig. 3A) that arch over the peristomial area. When moved towards the mouth, these spines cover almost all of the peristome except for small areas in the adjacent ambulacra, but can be flexed outwards from the peristome to admit larger food particles. These spines have the longest ciliary bands on the urchin. The spines themselves can be longer than 1 millimeter, and the bands stop short of the tip (Fig. 2H).

*Spine histology*

Spines are made up of a stereom shaft extending distally from the base. The base articulates on a tubercle to form the ball-and-socket joint typical of most echinoids. A ring of muscles surrounds the base so that the spine is free to swing in any direction. The shaft is made of be-

tween 6 (in miliaries) and more than 15 (in larger primaries) parallel ribs assembled in a cylinder and connected by a meshwork of struts between and internal to the ribs. Each spine is covered by a thin epithelium below which lie thin strips of nerve tissue between the stereom ribs of the shaft. These strips constitute the subepithelial nerve plexus of the spine (Fig. 3B, C). On diametrically opposing sides of the spine are two ciliary bands. The cells in these bands each support a single cilium that protrudes 20 or 30 μm beyond the level of the cuticle. The ciliary bands are 4 or 5 cells wide and create a slightly thickened, longitudinal ridge of epithelium (Fig. 3B, C). Most spines, whether primary or miliary, bear masses of conspicuous secretory cells between the stereom ribs. These stain brilliant purple in toluidine blue, and have a distinct granular appearance (Fig. 3C). The cells are absent from the proximal region of the spine (Fig. 3B), but increase in number towards the tip.

### Ciliary currents

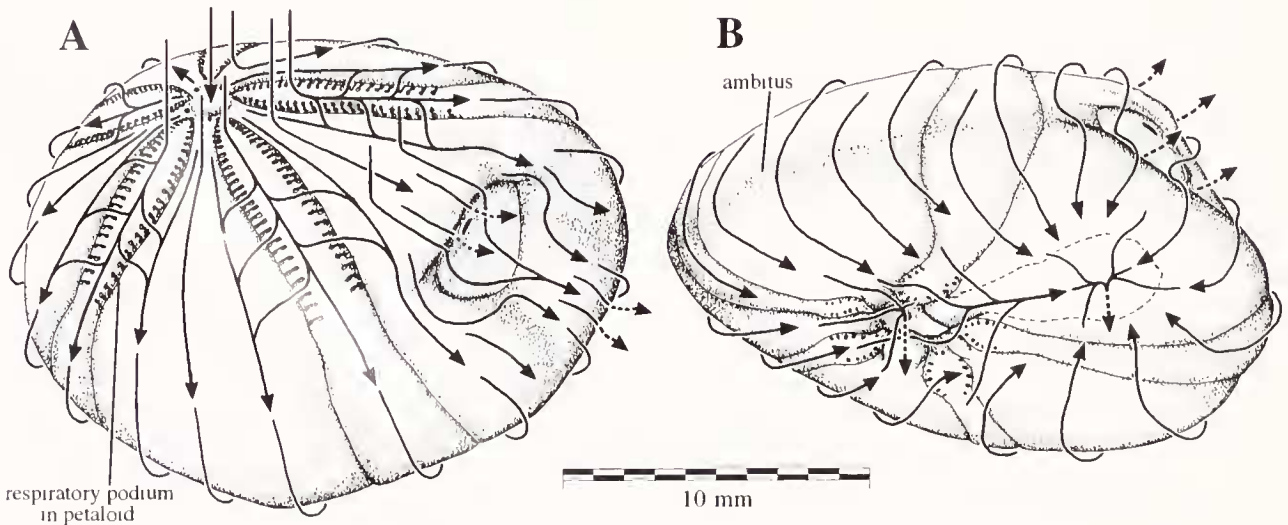
Currents generated by the ciliary bands on the spines flow between the spine shafts in a pattern common to all the specimens observed (Fig. 4). Fresh seawater can enter the spaces between the spines anywhere except places at which flow leaves the test, but the primary entry points of ambient water seem to be at the center of the aboral surface (Fig. 4A). Centrifugal flow then continues downward towards the periphery of the test (ambitus). Currents flowing down the centers of the ambulacra flow circumferentially for a short distance until they leave the petaloids by passing between the respiratory podia. Particularly strong flow is associated with the anal sulcus area, at which currents sweep across the periproctal plates and then along the sulcus to the ambitus. Some flow away from the test was observed at the edge of the hooded region just above the periproct, and at the ambitus below the periproct (Fig. 4A). However, the vast majority of the flow continues around the ambitus and onto the oral surface.

Anteriorly on the oral surface, the currents flow centripetally until they reach the mouth; then they converge and flow downwards away from the center of the oral surface. Posteriorly, the currents also converge, this time at a point in the posterior region of the naked zone. This area of convergence is characterized by additional downward flow away from the test. Strong currents of water that did not leave the echinoid at the peristomial region of convergence were seen to travel on either side of the peristome and then along the center of the naked zone directly towards the posterior region of convergence (Fig. 4B). Ciliary currents were never observed to augment the feeding process, and were in fact very active in com-

pletely removing particulate material from the test surface, and in providing continuous current over the respiratory podia of the petaloids.

### Feeding mechanism

*C. caribaeorum* feeds exclusively with its podia; spines and pedicellariae were never seen to aid in food-gathering or ingestion. Accessory podia first probed and then attached to particles of ambient sediment. There was no obvious selection for particular elements of the sediment, in spite of the abundance of bright pink or discoidal foraminifera. Phyllopodia frequently procured particles for ingestion, but the task of gathering food particles was clearly divided between accessory podia and phyllopodia. Although the accessories on the oral surface were more active in food-gathering, those on the aboral surface were also seen to draw in and pass particles around the ambitus to podia on the oral surface. A given particle was then manipulated towards the peristome, frequently by more than one podium at a time, until the phyllopodia and buccal podia could move the particle into the mouth. Once picked up by podia, particles were not agglutinated in masses or strings. Instead, each particle was handled separately until ingested. The circumoral spines moved downwards away from the peristome to allow passage of larger particles into the mouth. The mouth opening itself could be greatly enlarged through retraction of the peristomial membrane towards the peristomial edge, thereby allowing the particles to be swallowed. It was not clear how the act of swallowing was actually accomplished, although the peristomial membrane was equipped with liplike regions directly adjacent to the



**Figure 4.** Ciliary currents on *Cassidulus caribaeorum*. (A) Oblique aboral view from the posterior. (B) Oblique oral view from the posterior. Spines omitted for clarity. Solid arrows indicate flow towards test or within spine field; dashed arrows indicate flow away from test.



mouth that could aid in pushing the particles up into the esophagus as the mouth was drawn shut.

#### Particle analysis of Loblolly Bay sediment

The sediment of Loblolly Bay is a clean, calcareous sand of biogenic origin, consisting of about 30% coral fragments, 15% foraminifera, 15% mollusc shells, 5% echinoderm bits, 5% coralline algae, and 30% unidentifiable debris. Table I shows the distribution of different particle sizes, and their collective contributions to planar area and volume. For each size class the contribution to area is the sum of the squares of the mean dimensions expressed as a percentage of the total. Similarly, the volume contribution is based on the cubes of the mean linear dimensions.

#### Particle analysis of gut contents

When compared to predicted frequencies, particle frequency distributions in the gut contents of individual urchins differed significantly. However, no consistent pattern of variation was detectable, so the data do not point to particle size selection. The particle size frequency distributions for gut contents of 24 individuals ranging from 3.7 mm to 26.8 mm test length are shown in Table II. These data indicate that all urchins feed on similarly sized particles, and that the population as a whole is not selecting for any given particle size class or classes. *G*-statistics were calculated to test the significance of the observed and expected distribution of particle size frequencies in the pooled gut contents (Table III). The size classes below 500  $\mu\text{m}$  were represented in the gut samples by observed frequencies of less than 100. They were

pooled to avoid inflation of the *G*-statistic. Similarly, particles greater than 1600  $\mu\text{m}$  (1610.8–1987.4  $\mu\text{m}$ ) were pooled to make the test as conservative as possible. *G*-statistics were calculated for expected frequencies based on simple particle frequency proportions ( $G = 6427.245$ ,  $df = 12$ ), on contribution to area ( $G = 141.583$ ,  $df = 12$ ), and on contribution to volume ( $G = 1984.638$ ,  $df = 12$ ). All of the *G*-statistics are significant at the 0.01 level of probability ( $G = 26.217$ ).

Although statistically significant, the magnitude of the difference in *G* values suggests that particles are most probably collected according to the contribution made to area by each size class. The probability of getting a *G* value of this magnitude was tested by the stochastic simulation of 10,000 samples consisting of 20,514 particles. The range was 16.926 to 241.701 and the mean *G* for these simulations was 103.223 ( $\pm 44.714$ ), the median was 110.206, and the 95% confidence limits were 35.161 to 147.903. Among the 10,000 determinations of *G*, there were only 63 lower than 26.217, thus 99.37% of them would be ranked as "statistically significant."

#### Test allometry

Table IV presents the results of regression analyses for various test dimensions, using test length along the antero-posterior axis as the independent variable. The exponents for test width and test height do not differ significantly from 1.00, indicating that *C. caribaeorum* does not change shape as its size increases. In contrast, the exponents for width and length of the peristome indicate that the aperture increases more slowly than test length, and thus the opening becomes relatively smaller as the urchins increase in size.

#### Podial allometry

Results of regression analyses of podial disk diameters (dependent variables) and test length (independent variable) are shown in Figure 5. Separate analyses for the podia in ambulacra III, IV, and V showed nonsignificant differences, so the data were pooled. All exponents are significantly less than 1.00: as the test size increases, the podial disk diameter becomes relatively smaller. Within the observed size range of *C. caribaeorum* (up to 26.8 mm test length), the disks of the buccal podia are always slightly larger than those of the phyllopodia, but the two structures are otherwise indistinguishable. Although the accessory podia are similar in general morphology to the buccals and phyllopodia, they have markedly smaller disks. Moreover, this disparity in disk size becomes increasingly apparent as the urchin becomes larger.

#### Gut content allometry

Allometric analyses were performed on the numbers of particles in the gut, and on the mass of the gut contents

Table I

Frequency distribution of different sized particles in sand from Loblolly Bay (Anegada, B.V.I.), collected from small berm inhabited by *Cassidulus caribaeorum*. Data represent 5 pooled samples

Particle size class, $\mu\text{m}$	<i>n</i>	Proportion by frequency	Proportion by area	Proportion by volume
<500	1363	0.12615	0.01356	0.00391
500–600	458	0.04239	0.02205	0.01498
600–700	2715	0.25126	0.18257	0.13684
700–800	2601	0.24072	0.23286	0.20248
800–900	1766	0.16344	0.20308	0.19138
900–1000	980	0.09070	0.14078	0.15431
1000–1100	491	0.04544	0.08616	0.10432
1100–1200	174	0.01610	0.03663	0.04867
1200–1300	89	0.00824	0.02213	0.03291
1300–1400	69	0.00639	0.02001	0.03227
1400–1500	35	0.00324	0.01171	0.01968
1500–1600	26	0.00241	0.00994	0.01887
>1600	38	0.00352	0.01852	0.03838
Totals	10805	1.00000	1.00000	1.00000

Table II

Particle size frequency in the guts of 24 *Cassidulus caribaeorum* arranged in order of test length (TL)

TL (mm)	Particle size class ( $\times 100 \mu\text{m}$ )												Total	
	<5	>5	>6	>7	>8	>9	>10	>11	>12	>13	>14	>15		>16
3.7	0	0	1	0	0	0	0	0	0	0	0	0	0	1
4.3	0	0	0	3	0	0	0	0	0	0	0	0	0	3
4.3	0	1	1	5	14	9	1	2	1	0	0	0	0	34
4.9	1	8	11	19	16	6	3	2	1	0	0	0	0	67
5.4	1	10	23	23	12	7	0	0	0	0	0	0	0	76
6.7	8	28	38	38	13	10	2	0	0	0	0	0	0	137
7.0	3	4	48	47	24	17	5	2	1	1	5	9	6	172
8.3	5	26	77	59	40	10	4	7	3	0	0	0	0	231
9.2	6	27	58	72	65	63	31	14	2	1	0	0	0	339
9.3	2	31	101	77	69	40	15	10	3	2	1	1	0	352
11.3	8	18	99	115	79	65	27	14	3	2	2	11	10	453
11.3	14	8	125	131	98	47	33	16	5	24	14	13	21	549
12.3	15	39	151	150	98	68	47	17	11	18	4	11	10	639
13.1	31	25	188	191	105	42	28	13	23	23	12	12	11	704
13.4	13	14	152	185	139	83	30	16	8	27	25	13	1	706
15.2	8	22	234	260	238	108	64	27	14	9	16	12	31	1043
15.6	0	11	119	198	215	177	94	38	23	17	8	2	0	902
15.8	3	0	192	213	222	179	97	49	19	9	5	31	21	1040
17.3	5	33	137	273	303	244	164	71	49	27	17	9	1	1333
18.5	17	4	176	328	332	270	179	99	48	74	15	8	2	1552
20.1	22	26	434	507	362	236	274	79	52	37	23	12	7	2071
20.9	58	65	516	658	473	447	184	77	59	42	28	27	37	2671
22.6	34	49	513	652	620	414	239	46	51	36	21	13	8	2696
26.8	27	8	390	620	671	370	266	159	82	66	47	22	15	2743
Total	281	457	3784	4824	4208	2916	1787	758	458	415	243	206	181	20514

Table III

Observed and expected frequencies of different sized particles (with G-statistics) in the pooled gut contents of 24 *Cassidulus caribaeorum* shown individually in Table II

Midpoint of size class ( $\mu\text{m}$ )	Observed F	Expected F	Expected A	Expected V
250	281	2588	278	80
550	457	870	452	307
650	3784	5155	3746	2808
750	4824	4938	4776	4164
850	4208	3353	4166	3936
950	2916	1861	2889	3169
1050	1787	932	1767	2140
1150	758	330	751	998
1250	458	169	454	675
1350	415	131	410	659
1450	243	66	240	404
1550	206	49	204	387
1750	181	72	380	787
G (12 df)		6427.245	141.583	1984.638
P		$\leq 0.001$	$< 0.001$	$\leq 0.001$

Expected frequencies were calculated assuming random collection by these urchins using the probabilities of encountering the particles based on their frequency (F), and their contributions to planar surface area (A) and volume (V) of the surrounding sediment.

(dependent variables), and test length (independent variable). The results are shown in Table IV.

## Discussion

### *Roles of the external appendages in feeding and cleansing activities*

As in clypeasteroids, the podia of cassiduloids are clearly the "right equipment" for food-gathering (see arguments in Telford *et al.*, 1985, and Mooi, 1986b). The histology of cassiduloid podia is very similar to that of clypeasteroids, with well-developed sensory capabilities, secretory cells for provision of adhesive substances, and musculature for efficient particle manipulation toward the mouth (Fig. 1). However, cassiduloid podia tend to be larger than those of clypeasteroids, even in small forms such as *C. caribaeorum*. This is especially true for the greatly expanded tips of the phyllopodia found on cassiduloids. Our observations of gross morphology suggest that these are more reminiscent of those in regular echinoids, in which the disk diameter to shaft diameter ratio is about 2:1. In clypeasteroids, this ratio is closer to 1:1 (Mooi, 1986a). Clypeasteroids also have a greater diversity of podial types and a much higher total number of podia than cassiduloids. These additional differences

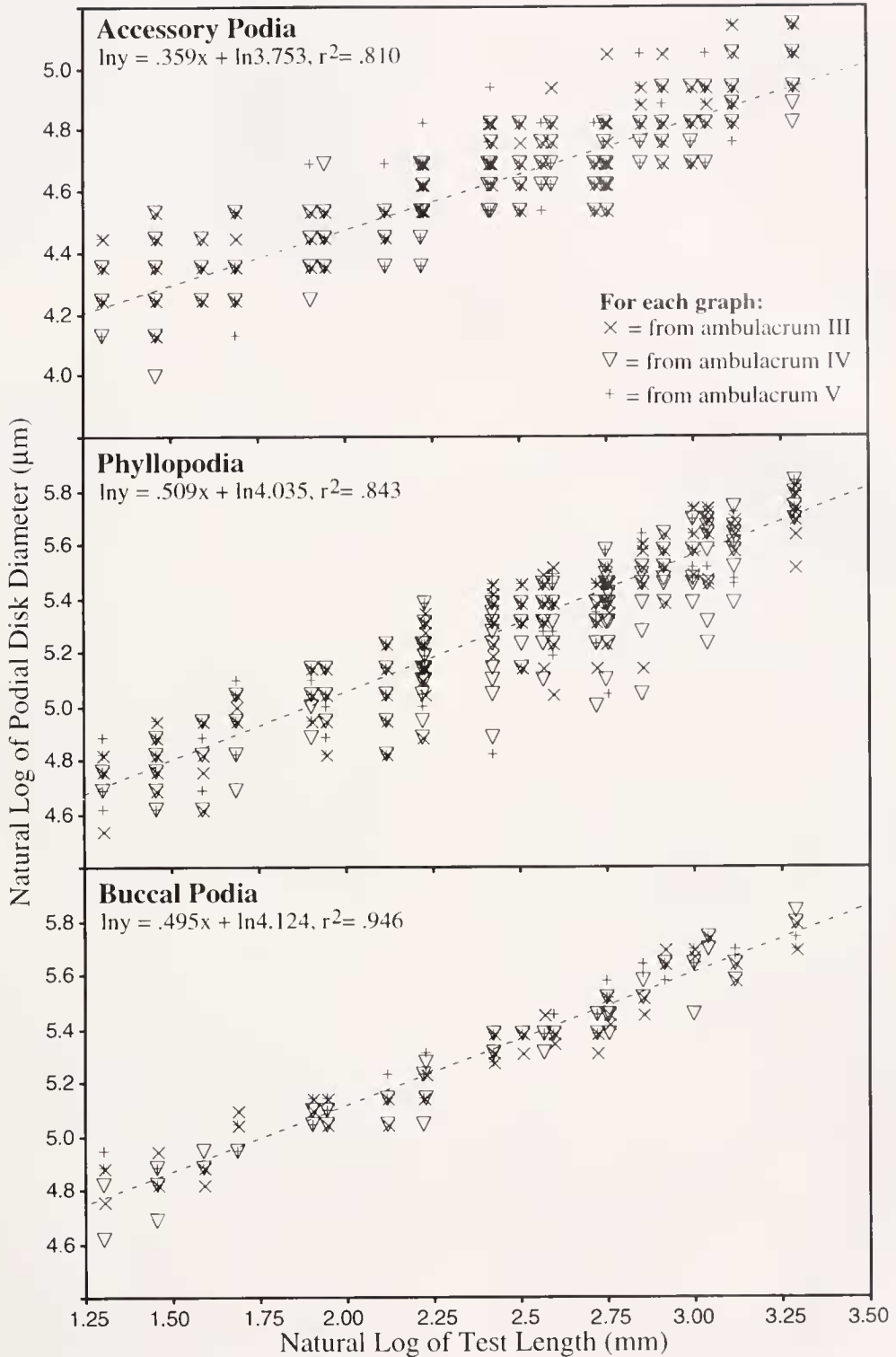


Figure 5. Plots and regression analyses of podial disk diameter against test length for each of the three nonrespiratory podial types in 24 specimens of *Cassidulus caribaeorum*.

are related to a radically different manner of feeding, and to differences in the size ranges of sediment particles that form the diets of these two echinoid groups. Clypeaster-

oids handle many thousands of particles at any moment during the feeding process, employing similar numbers of greatly miniaturized accessory podia to collect these

Table IV

Regression analyses of test dimensions and gut contents (dependent variables) against test length (independent variable, mm) in *Cassidulus caribaeorum* ( $n = 20$ )

Dependent variable	Exponent ( <i>b</i> )	Coefficient ( <i>a</i> )	$r^2$
Width (mm)	0.97	0.925	0.996
Height (mm)	1.04	0.404	0.988
Peristome width (mm)	0.56	0.530	0.927
Peristome length (mm)	0.54	0.434	0.959
Number of particles in gut	2.34	1.69	0.986
Mass of particles in gut (g)	2.71	0.002	0.982

particles (see evolutionary discussion below). Banks of podia of differing dimensions in different regions of the test work together to pass particles into food grooves where they are amalgamated into loose strings by the secretory and manipulatory action of specialized podia lining the grooves. Cassiduloids generally feed on coarser sands than clypeasteroids, and the entire complement of cassiduloid podia is capable of picking up only a few particles at a time. Cassiduloids lack food grooves, relying instead on enlarged phyllopodia that manipulate individual particles towards the mouth.

Histology and *in vivo* observations (Figs. 2, 4) of other external appendages of *C. caribaeorum* indicate that only the podia are used in food-gathering. This is completely consistent with what is known about feeding in other irregular echinoids. Although spines were once thought to function in feeding of clypeasteroids, this has been shown not to be the case (Telford *et al.*, 1985; Mooi, 1986b, c). Cassiduloid spines are likewise not employed in feeding. Even the circumoral bourrelet spines do not aid in the ingestion of particles, although they might help in keeping particles next to the peristomial membrane until they can be swallowed.

The easily observed, powerful ciliary currents of *C. caribaeorum* are strictly for cleaning and ventilation. In burrowers such as this species, the importance of ciliary flow cannot be overestimated. The hydrodynamic principle of continuity is evident in the way that fresh seawater is continuously introduced, largely at the apical region, to flow down around the test and then leave it in specific areas at the posterior and oral regions (Fig. 4). This principle plays an important role in keeping the test surface free of obstructing fine material and in meeting the need of the epithelium for dissolved organic nutrients and gas exchange. In addition, surface flow produced by cilia on the aboral primary and miliary spines is instrumental in maintaining flow over the respiratory podia in the petaloids (Fig. 4). Gladfelter (1978) noted the high respiratory rates of *C. caribaeorum*. We also noted that even slight drops in the flow of fresh seawater through the

sediment in which the echinoids were burrowed induced them to come to the surface immediately. Both of these observations suggest the importance of ciliary currents in alleviating such stress in the wild. Spines on both surfaces of *C. caribaeorum* are also capable of producing mucous secretions (Fig. 3), but their purpose remains obscure, except that a general cleansing function, such as that seen in spatangoids and some members of the clypeasteroid genus *Clypeaster*, seems reasonable (Telford *et al.*, 1987).

#### *The allometry of particle collection*

The allometric analysis of test dimensions indicates that as size increases in *C. caribaeorum*, the proportions of the test do not change. In contrast, podial diameters increase more slowly than test dimensions, so that the podia of adult urchins are relatively much smaller than those of juveniles. *C. caribaeorum* is a brooding urchin in which juveniles (less than 4 or 5 mm test length) are retained among the aboral spines of the adult females. When the juveniles are eventually released, they inhabit exactly the same sediment as the adults, feeding on the same array of particle sizes. The similarity in podial dimensions in different-sized urchins should not, therefore, be surprising. The size of the peristomial opening becomes relatively smaller as urchins increase in size, and this too should be expected if the urchins are ingesting similar-sized particles.

Comparisons of gut contents in urchins of different sizes show that they do, in fact, feed on similarly sized particles (Table II). No consistent differences were found in the linear dimensions or in the masses of particles in different sized urchins. Allometric analysis (Table IV) showed that the mass of material in the gut increases less rapidly than the cube of the linear dimension ( $b$  values of 2.34 for number of particles and 2.71 for mass of particles vs. test length is significantly different from 3,  $P = 0.005$ ), suggesting either that the gut in *C. caribaeorum* does not scale isometrically with body volume, or that the animals do not keep the gut full at all times.

Gladfelter (1978) considered *C. caribaeorum* to be a somewhat selective feeder. Selectivity by deposit-feeding organisms results in a consistent, statistically significant, difference between the particle size frequency distributions of ingested material and of the surrounding sediment. This may arise passively from constraints in the collection mechanism, as it does in sand dollars (Telford, 1990), or as a consequence of active sorting and selection, as in some tentaculate worms (Jumars *et al.*, 1982). Most data on deposit feeding come from sieve analyses, in which the mass of material in each size class in the gut is compared to the distribution in the sediment. The unstated underlying assumption is that particles are available in proportion to their mass representation in

the sediment. In a stochastic simulation of deposit feeding in clypeasteroids, Telford (1990) showed that the probability of a foraging podium contacting a particle of any particular size class depends on the contribution that class makes to the planar (projected) area of the sediment, not to its volume or mass contribution. In addition, our data consist of frequency distributions, not masses. Conventional deposit feeding data consisting of the continuous variable, mass, are best treated by analysis of variance. Frequency data are best analyzed by the *G*-statistic or log-likelihood ratio test.

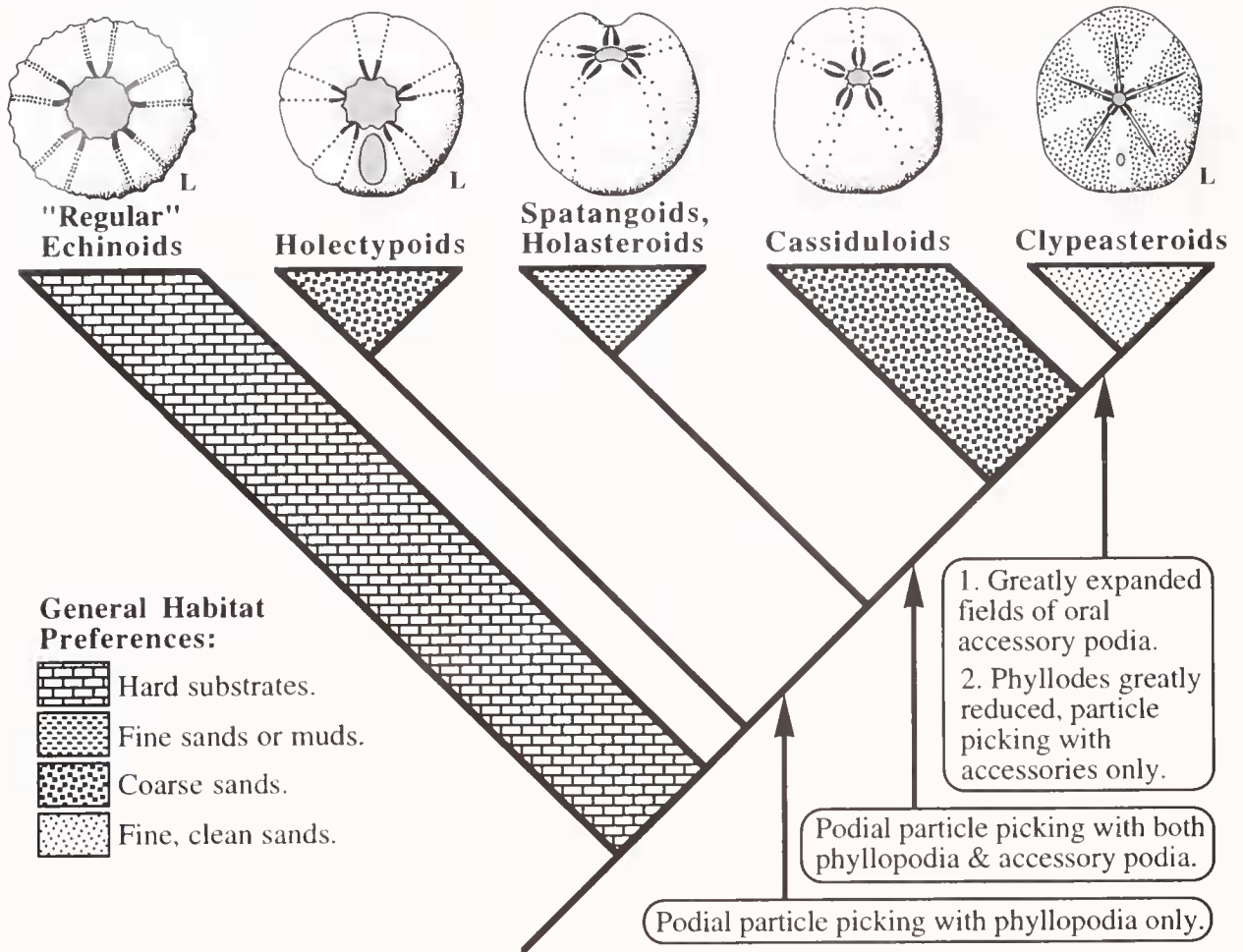
According to our analysis, all gut samples differ significantly from the native sediment and from each other (Table II). Thus we have one requirement to establish selectivity, that is, statistical significance. But there is no consistent pattern of preferred size classes and no correlation of ingested particle sizes with test dimensions. Although all of the individual and the pooled gut contents exhibit a statistically significant difference from the sediment, the sheer magnitude of the *G* values (Table III) indicates that particle collection probably occurs in proportion to their areal representation rather than to volume or simple frequency. Making this assumption, we examined the distribution of *G* values by stochastic modeling: we analyzed 10,000 randomly drawn samples to compare with the observed pooled gut contents. Our stochastic model suggests that randomly drawn particle arrays will usually appear to be significantly different to the parent sediment and that caution is therefore needed in the interpretation of these *G*-tests. Lacking persuasive evidence to the contrary, we conclude that *C. caribaeorum* is probably a non-selective feeder: regardless of test size, all individuals appear to draw particles randomly from the sediment.

#### *Evolution of podial particle picking*

Elucidation of the feeding mechanism of a cassiduloid helps to fill in some of the gaps in our knowledge of the evolution of the PPPM described by Telford *et al.* (1985) and Telford and Mooi (1986). The major events in the history of this mechanism are depicted in Figure 6. Both the "regular" echinoids (a paraphyletic assemblage) and the earliest of the Irregularia, the holectypoids, had a well-developed, upright Aristotle's lantern that could be protruded to rasp at food materials positioned directly below the mouth. The accessory podia of regular echinoids are primarily food-holding, sensory, or locomotory structures. In some taxa, adoral concentrations of podia adjacent to the peristome help the echinoid maintain its position on hard bottoms exposed to waves and currents. These podia do not act as food-gathering organs. Although holectypoids seem to have inhabited coarse sands, the PPPM probably did not play a major role in food-gathering in these forms either. After a care-

ful analysis of the podial pore arrangements and overall morphology of *Holectypus*, Smith (1984: p. 106) states that it "fed using only its lantern to gather food, scooping up bottom sediment fairly unselectively. . ." The phylogenetic position of *Holectypus* (Smith, 1984) suggests that this behavior represents the plesiomorphic condition for the holectypoids. However, the extant, derived holectypoid *Echinoneus* has secondarily lost the lantern. According to Rose (1978: p. 302), sediment particles were "picked up by the nearest tube-feet and passed along the ambulacra to the mouth." Therefore, the PPPM apparently plays a major role in the feeding mechanism of *Echinoneus*. This represents a striking convergence with other lanternless Irregularia. In the spatangoids and holasteroids, which generally inhabit very fine sands or mud, the particle-picking role is restricted to the penicillate phyllopodia, which are capable of collecting large numbers of particles with each application of a podium to the sediment (Nichols, 1959; Smith, 1984).

Although the particle-gathering function of the phyllopodia is still very important to cassiduloids, they were among the first echinoids to employ accessory podia as particle-gathering organs. *C. caribaeorum* illustrates this capability very well in its use of accessories to both probe and pull particles towards the test and pass them from podium to podium towards the mouth. The elaboration of the PPPM from this earlier cassiduloid pattern involved increasing the number of accessory podia in contact with the sediment. The extinct oligopygoids (not shown in Fig. 6) had already multiplied the number of accessory podia in each ambulacrum through an elaborate plate compounding system (Kier, 1967), thereby increasing the role of the accessories in the PPPM. The clypeasteroids, in breaking the "one-podium-per-plate rule" (Mooi, 1990a), were able to vastly increase the number of accessory podia. Each accessory could operate as part of the PPPM by picking up one or two particles at a time. In some clypeasteroids, there may be up to 160 food-gathering podia per mm<sup>2</sup>, giving some individuals hundreds of thousands of such podia on the oral surface, all acting as parts of miniature "bucket brigades" as they pass particles towards the mouth. Particularly in species of Mellitidae, accessory podia can cover 40% of the oral surface (Telford and Mooi, 1986). In these forms, accessories can be elaborated into several types, such as the long and short barrel-tipped podia typical of *Leodia* and *Encope*. Mooi (1986b) distinguished between accessory podia from the aboral surface and ambitus, and barrel-tipped podia from the oral surface, but the latter undoubtedly represent modifications of the former. In the scutelline clypeasteroids, the PPPM has become a sophisticated operation that relies exclusively on accessory podia and their derivatives to gather particles and pass them into the food grooves (Telford *et al.*, 1985).



**Figure 6.** Phylogeny of podial particle picking in the major extant clades of Echinoidea. Arrows indicate positions of events in evolution of particle picking listed within boxes. In small figures of echinoids, mouth and anus are shaded, phylloporia are shown in solid black, accessory podial pores are indicated by dots in ambulacra, "L" indicates taxa with an Aristotle's lantern in the adult. Phylogeny after Smith (1984).

In most clypeasteroids there is a small set of slightly enlarged podia [which Mooi (1986b) called "large food groove podia"] adjacent to the peristome, but just distal to the buccal podia. These podia are similar in overall morphology and histology to other food groove podia, but are about twice their size. Large food groove podia are not involved in particle collection; rather, they help guide strings of aggregated food material into the mouth. Phelan (1977) suggested that the phylloporia of cassiduloids were homologous with the entire podial field on the oral surface of clypeasteroids. However, accessory podia are modified into phyllopodia during the ontogeny of cassiduloids, a process also responsible for the differentiation of the large food groove podia of clypeasteroids. The rest of the accessory podia in the expansive ambulacral fields of clypeasteroids do not undergo this modification. These developmental patterns, as well as the overall anatomy and arrangement of the phyllopodia in cassiduloids, indicate that their phylloporia are not homol-

ogous with the entire podial field of clypeasteroids. Because they are intermediate in size between the buccal podia and accessory podia, and because they form by differentiation of accessory podia associated with the rest of the ambulacrum, the large food groove podia of clypeasteroids are the most likely candidates for homologues of the phyllopodia of cassiduloids. The large food groove podia of clypeasteroids probably represent a greatly reduced phylloporium (Fig. 6). If so, then this reduced phylloporium is homologous with the phylloporium of spatangoids and cassiduloids.

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